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# BOTANICAL GAZETTE

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## A STUDY OF REDUCTION IN *OENOTHERA* *RUBRINERVIS*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY III

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(WITH PLATES I-III)

The present contribution is a statement of some of the results obtained in the cytological study of *Oenothera Lamarckiana* and its mutants. Since these results have a more or less direct bearing on a wide range of fact and theory in various fields, their full discussion is reserved for a future time. The facts presented in this communication will be taken almost wholly from the study of *O. rubrinervis*, one of the most vigorous of the mutants. Other papers will be presented later, giving the further evidence upon which the conclusions of this paper rest, and attempting to indicate their bearing on the general problems of cytology and variation involved.

### Material

The plants from which the material for these studies was obtained were grown from pedigree seeds of DEVRIES, the purity of these cultures being further proven, in some cases, by carrying on the pedigree for another generation before collections were made. The results of these cultures, which are still being carried forward to later generations, will be published at another time in connection with an account of other studies on variation and hybridization in *Oenothera*. In this way it is hoped, if possible, to correlate the cytological data with the work in hybridization and variation. It seems to the writer that only by thus combining cytological with experimental studies is an explanation of the peculiar and remarkable phenomena of variation exhibited by the *Oenotheras* to be reached.

The cytological studies presented here will be confined chiefly to the phenomena of synapsis and reduction in the pollen mother cell. Various forms have been studied, a complete series of stages being obtained in some forms and a partial series or only a few stages being examined in others. The forms investigated include (1) *O. rubrinervis*, (2) *O. Lamarckiana*, (3) *O. gigas*, (4) *O. nanella*, (5) *O. biennis cruciata*, a variety of the European *O. biennis*, (6) both *O. lata* (see 12) and *O. Lamarckiana* from the  $F_1$  of *O. lata*  $\times$  *O. Lamarckiana*, and (7) plants resembling *O. gigas*, from the  $F_1$  of *O. lata*  $\times$  *O. gigas*. Preliminary reports have already been made upon some of these studies, in various connections (11, 12, 13, 14, 15). Reference will be made to some of these results later.

The material from each individual was collected separately in nearly all cases, in order to observe possible individual differences in the same race, either in the number of chromosomes or in other cytological features. I am indebted to Mr. C. H. SHATTUCK for making a number of these collections. The material for the study of *O. rubrinervis* was obtained from a number of individuals grown in two different seasons and representing several strains derived from the same original pedigree. Sections were cut from six of these, and it may be stated here that in *O. rubrinervis* no individual differences were discovered, either in the number of chromosomes, which was 14 in all cases, or in any other features. In some of the other mutants, also, a number of individuals were examined. It was found necessary to reserve the account of *O. gigas*, which presents several features of special interest, for a separate paper. A preliminary report on this form has already been made (14, 15).

For various reasons, *O. rubrinervis* was chosen as the most favorable form for a thorough study of synapsis and reduction. The nuclei and chromosomes of *Oenothera* are small, and for this reason the selection of the most favorable type for study is a matter of some importance. In *O. rubrinervis* the pollen mother cells, although they vary much in size, are usually considerably larger than in *O. Lamarckiana*, the nuclei being also proportionately larger. The reason for this will be explained later. The chromosome number being low in most of the forms ( $2x=14$ ,  $x=7$ ), they can be counted without any difficulty, notwithstanding their small size. Another

notable advantage in comparing this with other studies in reduction is in the shape of the chromosomes, which are globular or somewhat oblong or cylindrical in most stages of mitosis, and are never greatly elongated or looped. For this reason it is a comparatively easy matter to obtain accurate counts of the chromosomes in the pollen mother cells of any of the forms. This shape is also found to be very advantageous in a study of the events of reduction following synapsis. The appearances are clear and easily interpreted, in striking contrast to the forms with long twisted chromosomes, such as have been made the basis of many of the studies on reduction in plants.

On the other hand, the somatic nuclei and chromosomes are very much smaller, and in metaphase the latter are elongated and looped, making it impossible to count them with the same degree of accuracy. Some of these appearances have already been described elsewhere (12, p. 19). Thus while it was found that the chromosomes could be counted almost equally well in pollen mother cells of all the forms studied, *O. rubrinervis* was found to be especially favorable for the investigation of reduction phenomena, especially the events of synapsis and the prophases of the heterotypic mitosis. The account given here will refer throughout to *O. rubrinervis*, with occasional comparisons with other forms. Later papers will take up these other forms in detail, in so far as this is necessary after the account presented here. Special attention will be given at that time, in particular, to the later stages, beginning with the telophase of the heterotypic mitosis, and also to the interesting conditions in some of the hybrids. The detailed account in *O. rubrinervis* will not be carried farther than the metaphase of the heterotypic mitosis, at which time the essential events have already taken place.

### Methods

The usual methods of cytological technique were employed, various chrom-acetic and chrom-osmo-acetic solutions being tried until satisfactory fixation was obtained. The thickness of the sections varied from 4 to 10  $\mu$ . The latter thickness was found most favorable for counting chromosomes, because it is somewhat greater than the diameter of the nuclei, many of which in such sections were therefore uncut. It is possible to determine easily whether a nucleus has been cut by the knife by examining it in low and high focus. The

chromosomes in such uncut nuclei can then be counted with absolute accuracy, either in the prophase of the heterotypic mitosis before the disappearance of the nuclear membrane, or in the telophase after the walls of the daughter nuclei are formed. In nearly every individual examined, large numbers of such cases, all yielding the same result, were counted before the number was finally determined upon. The chromosomes could also be counted in certain positions on the spindle, particularly in anaphases, but in metaphase they were usually too closely grouped to allow of satisfactory counting.

In the second division, particularly in the forms having seven chromosomes as the gametophytic number, the chromosomes could be counted with certainty in almost any stage of mitosis. The thinner sections were used chiefly in the study of spirem and synapsis stages, although here also the comparatively short length of the thickened spirem frequently made it advantageous to study uncut nuclei in which the spirem could be followed throughout its length.

Of the various stains Heidenhain's iron-hematoxylin was found to be superior for chromosome counting and for clear differentiation of chromatic structures in nearly all stages of synapsis and reduction, safranin-gentian being used occasionally for comparison or for differentiating particular cytoplasmic structures. Orange G was also used with the iron-alum stain for bringing out clearly certain special features, particularly the protoplasmic connections between mother cells, which will be described later.

### Description

#### EARLY STAGES

Some of the very early stages of the anthers, previous to the formation of mother cells, have been studied particularly with the purpose of tracing the origin of the bodies which were called heterochromosomes in my first paper. The provisional use of the name was based on the very close resemblance of these bodies to the chromosomes in appearance, and their frequent presence close by, or in some cases apparently attached to, the heterotypic spindle. They were not stated to pass undivided into one of the daughter nuclei, as misquoted by TISCHLER (32), but to remain outside in the cytoplasm where they gradually disappear. The study of their early history

shows that no line of distinction can be drawn between them and the large body readily recognized as the nucleolus. They are then smaller nucleoli, not differing essentially in origin from the single larger body which is almost constantly present in the mother cell during synapsis and prophase, but diverging from the latter somewhat in their later history.

In the earliest stages studied, the young meristematic cells of the anther primordia are very small (*figs. 1, 2*), and the tissues are wholly undifferentiated, except the epidermal layer. Usually several smaller nucleoli are present in each nucleus of the meristematic cells, in addition to the larger nucleolus. Compared with the cells of the anther wall when they are no longer meristematic, the smaller nucleoli of the former are about the size of the nucleoli of the latter, which are approximately equal in size. There is nothing in the latter corresponding to the larger nucleolus of the meristematic cells. Probably afterward one of these nucleoli enlarges as the cell increases in size, or it is possible that the nuclei of meristematic cells are always derived from previous ones which already possess a large nucleolus.

Chromatic staining bodies are also found closely appressed to the nuclear membrane in all the meristematic cells (*figs. 1, 2*). This tendency for chromatic material to accumulate on the nuclear walls gives these nuclei a characteristic appearance. These bodies often appear like a thickening of the membrane itself.

At the next stage studied many cell divisions have taken place, and the sporogenous, tapetal, and wall tissues have been differentiated. The sporogenous cells have increased enormously in size, and form a single row in longitudinal section down the center of the anther, the walls of these cells being especially thickened and distinct (*fig. 3*). The cells of the surrounding tapetal layer have also increased greatly in size and are distinctly marked off from the sporogenous row. In the sporogenous cells the nuclei (*fig. 4*), though much increased in size, have not increased in proportion to the cytoplasm. The large nucleolus, much larger than in the earlier stage, is now a conspicuous object in the nucleus. Smaller nucleolar bodies are almost invariably present, but masses are no longer found attached to the nuclear membrane. (The characteristic masses, however, may remain for some time attached to the nuclear walls of the tapetal cells).

*Figs. 5-10* are from drawings of other nuclei at this stage of development. In the majority of cases one or two smaller nucleoli occur in addition to a single large one, but rarely (*fig. 6*) two large nucleoli of equal size may be found; and very frequently the number of small bodies, of equal or unequal size, may be greater, reaching as many as five or six. *Figs. 5, 7, 8, 9* show these in various stages of fusion with each other and with the large nucleolus.<sup>1</sup> They are thus not in any sense autonomous bodies. It appears that usually these fusions take place until only one large nucleolus and one or two smaller ones are present during synapsis and diakinesis. But occasionally the fusions do not take place, and several of these bodies may then be present in the later stages. The number of these nucleoli finally present depends, then, largely upon the amount of fusion which has previously taken place between them. In the later stages one large nucleolus is almost invariably present and usually a smaller one bearing a certain proportion to the larger in size, though the latter may vary in size and number as already stated. There is usually a clear area around the large nucleolus, as in the earlier stage, and threads of the reticulum may or may not cross this and appear to be attached to the nucleolus (*fig. 4*). The reticulum of the cytoplasm usually stains rather more deeply at this time than that of the nucleus. It may as well be stated at this time that in the resting nuclei of the pollen tetrad and in the nuclei of the nearly mature pollen grains of *Oenothera* one finds (*fig. 11*) the same condition of the nucleoli as in the mother cells, namely, usually one large and one small nucleolus bearing a rather definite size relation to each other, with sometimes additional small ones.

The sporogenous rows are differentiated from the tapetum by the greater growth of the cells, nuclei, and nucleoli of the former. At the same time they are distinctly marked off by the formation of a continuous thickened wall between tapetum and archesporium (*fig. 3*). It is obvious that as the cells and nuclei increase in size, the nucleolus grows also. Up to the time of synapsis the mother cells usually form a compact tissue, but about this time the cells begin to

<sup>1</sup> Miss NICHOLS (21) figures what are in all probability stages of fusion of large and small nucleoli in *Sarracenia* pollen mother cells, but interprets them as a budding-off of small bodies from the nucleolus. The budlike attachments to the nucleolus frequently observed by other authors are doubtless to be explained in like manner.

break apart at the corners where they meet, and before diakinesis is reached they are completely rounded off and independent, or they frequently remain connected with other mother cells only at the ends. In the meantime the cavity of the loculus grows rapidly, so that the mother cells, in normal development, usually lie loose in the cavity.

The events of synapsis and reduction usually go forward simultaneously throughout a flower, with comparatively little variation in the different parts of the same loculus or in the different anthers of a flower. In one flower, however, wide variation was found in different anthers, but comparative constancy in the loculus. One anther of this flower was in synapsis, another in diakinesis, another in metaphase of the heterotypic mitosis, and in still another some of the mother cells had completed the second mitosis. No abnormalities in the cytological condition of this flower were observed.

#### SYNAPSIS

After the stage described in *fig. 4*, the nucleus increases greatly in size, but without an appreciable increase in the size of the cell. The single row of sporogenous cells divides, so that there are usually two rows of pollen mother cells. Occasionally three or more mother cells are found in the cross-section of a loculus. In general there are fewer divisions than in the other forms, and this is at least one of the reasons why the mother cells are on the average larger than, for example, in *O. Lamarckiana*.

The resting nucleus of the pollen mother cell increases in size and begins to show signs of approaching synapsis. *Figs. 12, 13, 14* show stages in the beginning of this process. A number of these stages were found—although they are uncommon—in the same sections with regular synapsis stages. In some cases they occurred side by side with mother cells in which the synaptic knot had already been formed. A complete series of stages may be found in the same section, from the beginning of contraction to the formation of a close synaptic ball. The cytoplasm in these cells shows no contraction whatever, but is perfectly fixed. For this and other reasons there can be no doubt that this is a real contraction stage, leading to synapsis, and not a result of imperfect fixation, as one might judge on first examination.

That these nuclei are going into synapsis and not coming out is shown by several features: (1) the extremely delicate character of the threads, like those of the resting nucleus; (2) the fact that the periphery of the reticulum as it contracts frequently preserves perfectly the curved outline of the nuclear wall (*fig. 12*); (3) immediately after synapsis the thread is somewhat shorter and thicker than previously and appears to be continuous, while in the earlier contraction stages we still have the appearance of a reticulum (*fig. 13*).<sup>2</sup> As the contraction progresses, the threads are gradually rearranged from an anastomosing reticulum to a very long and continuous delicate thread. The exact manner of this rearrangement could not be observed, but one finds many transitions (*fig. 14*) from the anastomosing reticulum of the resting nucleus to the closely coiled and apparently continuous spirem of the synaptic knot (*fig. 15*). The contraction may take place from one side of the nucleus, leaving the reticulum attached for a time to the nuclear membrane at one point (*fig. 13*), or it may take place simultaneously from all sides (*fig. 12*). A few threads of the reticulum usually remain attached for a time to the nuclear membrane while the contraction is going on. These are drawn in finally as the synaptic ball becomes more compact.

The small number of these intermediate stages found indicates that they are passed through rather rapidly, the frequency of the occurrence of synapsis stages indicating, on the other hand, that this condition is of considerable duration.

No indication of a doubling or pairing of the threads during these intermediate contraction stages could be observed, though they were carefully searched for. Moreover, in the earliest stages of the synaptic ball the thread appears to be as thin and delicate as in the reticulum, which does not favor the view that a pairing has taken place. The evidence, then, so far as it goes, is decidedly not in favor of a pairing.

During these stages the nuclear membrane is often indistinct, making it difficult to define accurately the limits of the nucleus. The

<sup>2</sup> This explanation assumes, of course, that the synapsis stages themselves are normal and not due to artifact, as I presume all cytologists will now agree, although SCHAFFNER (29) apparently still entertains some doubt on the subject. The regularly coiled arrangement of the thread in the synaptic ball appears to me to be one of the best arguments against this stage being an artifact. Evidently a rearrangement of the threads is going on as contraction proceeds.

same condition is observed during synapsis, which is found in the same sections. In places the nuclear membrane has either disappeared or is too delicate to be observed. The cytoplasm, however, retains the original outline of the nucleus. MOTTIER (20) has apparently observed similar conditions of the nuclear membrane at this time. In some cases it is ruptured and a portion of it is actually carried inward with the nuclear reticulum at the beginning of the contraction (*fig. 12*). One is tempted to explain this as an artifact; but that this is not the explanation is shown by the considerations already mentioned. The explanation appears to be that as contraction proceeds a portion of the nuclear membrane may be torn away and carried inward attached to the threads. Frequently in these stages one finds the nuclear membrane present on one side of the nucleus but invisible elsewhere. This is the case in *fig. 12*, although the membrane was drawn as though complete. Observations of other nuclei bear out this interpretation, the nuclear membrane being clearly visible in some cases attached to portions of the reticulum which have contracted far away from the original position of the nuclear wall. In the late prophase, when the definitive chromosomes are formed, a distinct and perfect nuclear membrane is invariably present, so it would appear that in such cases as those just described a new membrane is afterward formed.

Mention must now be made of the chromatic staining material of the nucleus during these stages. The nucleolus is frequently, though not always, included within the synaptic knot. There is a tendency for other dark-staining bodies to accumulate near the periphery of the nucleus (*figs. 12, 14*); as contraction proceeds these are swept in by the reticulum. The exact relation they bear to the threads is not known. In some cases they appear, in the later stages of contraction at least, to form a part of the threads themselves, in other cases they appear to be merely inclusions in its coils. These bodies show no constancy in number, size, or shape. As the spirem takes on the appearance of the synaptic knot, they are still found in its meshes, and portions of the thread itself may also stain darkly, suggesting a solution of a part of their substance and its transfer into the thread. Even when the greater part of the spirem is completely decolorized certain portions of it retain the stain. This appears to be partly

due to the denser aggregation of the spirem in these regions, but in some cases it is evidently due to the presence of bodies which retain the stain and appear to be giving up the stainable part of their substance to the spirem. These bodies are evidently not the prochromosomes found by OVERTON (22) in certain dicotyledons, nor are they the gamosomes of STRASBURGER (30, 31).

Just the relation these bodies sustain to the spirem is not easy to determine. From *figs. 12* and *14* it is evident that they are at first small "nucleoli" caught in the contracting reticulum, but quite independent of it. Later they appear to give up a portion at least of their material to the spirem, finally disappearing as independent bodies. Usually, however, at least one of these bodies remains independent, and appears in synapsis and diakinesis as a small nucleolus bearing a definite relation to the size of the large nucleolus, being about the size of a chromosome. These bodies are usually free in the nuclear cavity (*fig. 15*). A certain depth of stain is required for demonstrating them during synapsis, for they usually decolorize more quickly than the large nucleolus. With a favorable stain they are found to be of strikingly uniform occurrence at this time. A plasma stain such as orange G may be used with advantage to demonstrate their presence. The uniformity in their occurrence is so great that for some time they were thought to be constant in size and number. With the demonstration of their inconstancy and their origin we have chosen to call them merely small nucleoli, as there appears to be no sufficient reason for another name. The (large) nucleolus disappears with great promptness immediately after the nuclear membrane breaks down, only persisting for a time in a few rare instances. In no case has fragmentation of the nucleolus, previous to its disappearance, been observed, although the presence of deeply staining globular bodies occasionally found near the periphery of the cytoplasm might be accounted for in this way. The mass of the latter, however, is sometimes greater than that of the nucleoli. The smaller nucleoli persist and are frequently found close by the heterotypic spindle. They may also be found on the homotypic spindle (*fig. 41*). Apparently they never reenter a nucleus, but remain in the cytoplasm until they finally disappear. These bodies have been found showing the same behavior in all the forms studied.

## POST-SYNAPTIC STAGES

Synapsis lasts for a comparatively long time, as shown by the frequency of its occurrence in the material sectioned. During this time the spirem shortens and thickens and then begins to arrange itself more loosely in the nuclear cavity. This shortening and thickening is progressive (*figs. 16-18*) and apparently continues for some time. During these stages the thickness of the spirem may be nearly uniform throughout, or it may vary greatly, giving a moniliform appearance, or the spirem may appear irregularly constricted at varying intervals. In other cases, with a certain depth of stain it is seen to be composed of lighter and darker areas more or less regularly alternating. Portions of the thread may appear homogeneous or may show the lighter and darker areas, according to the depth of stain (*fig. 17*). In more deeply stained nuclei, such as *fig. 16*, the thread appears homogeneous throughout. These darker areas are the chromatin discs or chromomeres of various authors; and they give the thread a very characteristic appearance. During this well-defined stage the greatly thickened spirem is loosely distributed in the nuclear cavity. Deeply staining bodies still appear attached to or enmeshed in the coils of the thread.

At this time one finds undoubted indications of parallel threads. When represented by camera drawings in one plane the evidence for this is not so satisfactory as in the original preparation, but there is no doubt of their occurrence. As already stated, in the earlier stages previous to and during synapsis, parallel threads could not be observed, and it has not been determined whether they were really absent or whether the failure to observe them was due to their extreme delicacy. Hence it cannot now be stated whether they have arisen through an approximation of spirems at an earlier period, or through a split in the single continuous spirem. This matter will be discussed later.

Following this stage a second well-marked contraction takes place (*figs. 18, 20, 21*), apparently quite as typical and constant in its occurrence as the first contraction stage, which is ordinarily identified as synapsis. This contraction is of much shorter duration, however, and entirely different in appearance, owing to changes which the thread has undergone since synapsis, resulting in a great amount of shortening and thickening of the spirem. MOTTIER (20) has recognized this second contraction stage in *Podophyllum*, *Lilium*, and

*Tradescantia*, though he formerly thought it resulted from bad fixation; and it appears to have been observed also by FARMER and SHOVE (10). MOTTIER states that in these forms there is but little shortening of the spirem between synapsis and segmentation into chromosomes. In *Oenothera*, on the contrary, as is evident from a comparison of *figs. 15 or 16* with *22*, a very considerable amount of shortening as well as thickening of the spirem takes place during this interval. During the second contraction the paired threads apparently fuse, and further shortening of the (from now single) thread results in an enormous amount of thickening of the spirem, so that when it uncoils from this second contraction it has approximately the thickness of a chromosome and exhibits only a few loops. It can then frequently be traced through nearly its whole length. At this time there is a great amount of variation in the thickness of different parts of the spirem, as seen in *figs. 22 and 23*. *Fig. 19* is a portion of the spirem at this period, drawn with a higher magnification. It shows the chromatic bodies, which vary in size, imbedded in the linin substratum. As to how far two different substances are represented, I am at present unprepared to say.

#### DIAKINESIS

The single thick thread now segments transversely into 14 chromosomes, the sporophyte or  $2x$  number. At this time there is no indication whatever of a longitudinal split in the thread. Even when greatly washed out, the material of the chromosomes appears perfectly homogeneous, or if a granular structure is observable there is in its arrangement no indication of the previous split. At the time of this second contraction a pair of chromosomes is frequently observed separated from the spirem and apparently always lying with their long axes parallel and connected at one end (*figs. 20, 22*). This condition occurs very commonly, although in other cases the spirem is continuous throughout (*fig. 21*). In no case has more than one pair of chromosomes been observed to be thus precociously cut off in *O. rubrinervis*, though two such pairs have been observed in *O. lata* (see 11, *fig. 19*). In no case has a single chromosome been observed to be cut off in this manner, and apparently they are invariably cut off in pairs, that is, bivalent chromosomes are detached.

What significance this early separation of chromosome pairs may have is not known, but it appears that the later history of these pairs on the spindle can be traced. In the paper just cited (II), the writer wrongly identified them with the smaller nucleoli which persist by the heterotypic spindle. These chromosome pairs are frequently so closely approximated at the end opposite the end of actual connection as to give the appearance of a ring. It was thought that these rings by condensation (which actually takes place) were reduced to the size of these nucleolar bodies. The latter had the size and shape of chromosomes, and with a certain depth of stain invariably appeared hollow. These pairs are not condensed to rings, however, but to chromosome pairs of the ordinary *Oenothera* type.

The spirem at this time varies greatly in thickness in different parts, exhibiting constrictions and dilatations which indicate more or less clearly where segmentation into chromosomes will take place. This segmentation may happen while the spirem is still in the contracted condition (*fig. 25*), or after it has again uncoiled and distributed itself in the nuclear cavity (*figs. 24, 26, 28*), or before this uncoiling is completed. The segmentation appears to be in some cases nearly simultaneous (*fig. 24*); in other cases the segmentation is successive, as in *fig. 23*, where the spirem is clearly divided into three portions and the constrictions for the formation of the chromosomes are so far advanced that the number of chromosomes to be formed by each segment can already be foretold with practical certainty. The segmentation at this time is into 14 chromosomes, the sporophyte number. A large number of counts made at this time demonstrate the absolute constancy of this number in all the individuals of *O. rubrinervis* examined. It is possible, however, that individuals of this race may be found whose chromosome number differs from this number by one. This matter will be discussed later.

In every single case where the count could be determined with certainty it was shown to be 14. These counts were all made from sections 10  $\mu$  thick, and from nuclei which were uncut by the knife. The less numerous counts made in the multipolar stage of the heterotypic spindle gave invariably the same number. In this case all in a given cell were obtained by examining the adjacent sections. In all, hundreds of counts were made. In such nuclei as *figs. 26, 29, 30, 31*

there can be no possible doubt of the number of chromosomes present.

As already shown (*fig. 20*), one or in some cases more pairs of chromosomes may be cut off from the spirem before it undergoes segmentation, and frequently while it is still in the second contraction period. The exact method of origin of these pairs has not been observed, but they invariably, so far as observed, lie with their long axes parallel and connected at one end, from which it would appear that they were successive chromosomes on the spirem. In later stages, when the spirem has constricted into a chain of chromosomes arranged near the periphery of the nucleus, one or more pairs of chromosomes are found separated from the rest. Some of these have doubtless had the origin shown in *fig. 20*. Others appear to have originated later, as indicated in some of the figures, by successive chromosomes on the chain swinging around parallel to each other and thus pairing. Usually in diakinesis one or two such pairs are found, though occasionally there is no evidence of pairing. The highest number of pairs observed at this stage was five, with indications of pairing among the others (*fig. 29*); which is unusual. Later, in the multipolar spindle stage two distinct pairs are usually found in varying stages of conjugation (*figs. 35, 36*). A single case was observed (*fig. 37*) in which the fourteen chromosomes were all paired.

As the figures indicate, constriction of the spirem at regular intervals proceeds progressively until a chain of chromosomes is formed. When this has taken place, the chromosomes are at first several times longer than broad, and their margins have a very irregular, sinuous outline, like that of the spirem just previous to segmentation. They are not so long, however, that they can be twisted and looped in the confusing manner of many heterotypic chromosomes of plants. This is very gratifying in the study of these stages, since it permits a clearness of interpretation which would otherwise be unattainable. *Figs. 22 and 23* show the beginning of contraction, which has proceeded farther in *fig. 24*, leaving only the so-called linin connection between the chromosomes. The constrictions are all equivalent and the spirem thus segments into the sporophyte number of chromosomes and not into the reduced number of chromosome pairs. If successive chromosomes on the spirem are really the members of a pair, there is

nothing in the manner of segmentation of the spirem to indicate this. However, it is clear enough that one chromosome frequently swings around, as already mentioned, and pairs with its neighbor on the spirem. We do not really have, then, a transverse division of chromosome bivalents, but a separation of whole (somatic) chromosomes. Nothing has been found in the earlier stages which would correspond to the gamosomes and zygosomes of STRASBURGER, and even should a pairing of parallel threads during synapsis occur (a possibility which will be discussed later), the final pairing is between chromosome bodies which were lying end to end on a single spirem thread.

The linin connections during diakinesis appear to be merely the more finely drawn out portion of the spirem between the chromosomes. As condensation and contraction of the chromosomes progress, these linin connections become longer and more delicate (*figs. 31, 33*). The chromosomes become more dense and compact, being at first oblong-cylindrical (*figs. 24, 26*) and then more nearly globular or pear-shaped (*fig. 31*). Certain chromosomes sometimes undergo this contraction more quickly than others, as in *fig. 29*, and the different stages of this condensation may occasionally all be found in the same nucleus. In other cases the globular appearance is due to the position in which certain chromosomes happen to be lying (*fig. 34*).

#### HETEROTYPIC MITOSIS

During the prophase stages last outlined the cytoplasm usually possesses a more or less obscurely radiate appearance. A felt-work of fibrillae finally appears around the nuclear membrane. Later these fibrillae come to run tangentially to the latter, terminating in the cytoplasm, and by their aggregation in certain regions the multipolar spindle is formed. From this stage the fibers are rearranged to form the bipolar spindle, passing through conditions in which the spindle appears quadripolar or tripolar in section. In the meantime the nuclear membrane has dissolved and the chromosomes are found at first in a cavity surrounded by fibers which preserve the outline of the nuclear wall. Later they come in and become attached to the chromosomes. Usually the large nucleolus has vanished before this time, but occasionally it may still be seen (*fig. 35*). In *fig. 37* the small nucleolus is shown, which can very frequently be seen at this

time. *Figs. 36 and 37* are merely sketches of the spindle fibers to indicate their general direction. *Fig. 35* is an unusual case. A cone of fibers appears to have been formed on one side only of the nucleus. The fibers are coming in and finding attachment to the chromosomes. The large nucleolus is still present, as well as two smaller ones.

The most critical stages of reduction have now been described and the remaining stages will be taken up with less detail at this time, but will be presented in full in a later paper. The chromosomes are at first irregularly arranged on the heterotypic spindle. As already seen, during spindle formation many of the chromosomes are frequently separate and unpaired. The attraction between the chromosomes which leads to pairing is evidently weak, so that it is doubtful if any pairing takes place at metaphase between chromosomes which had not previously paired. On the other hand, chromosomes which have once paired, no matter how early, appear to remain together until their separation in the metaphase of the heterotypic mitosis. Hence probably in many cases the chromosomes pass to the poles of the heterotypic spindle without having previously paired with each other, that is, they were merely lying loosely in the equatorial region of the spindle in metaphase, so that it was largely a matter of chance which pole any particular chromosome went to. This is believed to be a matter of prime importance in determining the final result of the reduction divisions in *Oenothera*, and the nature of the distribution of chromatin elements which takes place. Its possible significance will be pointed out in the discussion. *Fig. 38* shows the chromosomes just being drawn into the equatorial plate of the heterotypic spindle. In the examination of thousands of spindles in about this stage, one usually finds the chromosomes spread out in several planes along the long axis of the spindle. Of course some of these are early anaphase stages in which the chromosomes have begun their journey to the poles, but the condition is seldom found where the chromosomes are arranged regularly in pairs on the spindle. The daughter chromosomes seldom advance toward the pole in a single plane, as is the case in so many forms, but are more or less irregularly strung out along the spindle in their passage to the poles. This is in striking contrast with their behavior in the homotypic mitosis.

Usually in the early anaphase of the heterotypic mitosis a longi-

tudinal split appears in the daughter chromosomes. This split does not stop short of one end, giving a V-shaped body as in many plant chromosomes, but usually passes right through, forming two independent bodies, which, however, remain paired in the telophase and occupy a great variety of positions in regard to each other. The homotypic chromosomes thus assume many of the characteristic shapes which are usually observed in the heterotypic chromosomes of other forms, such as X, Y, V, H, etc. The failure of the heterotypic bivalents to form these shapes is due partly to the weaker attraction between the members of a pair, but largely to a difference in their shape, each member of a pair being usually more rounded in the heterotypic and more elongated and rodlike during the stages between the two mitoses.

The telophase of the heterotypic mitosis is one of the best stages for counting the chromosomes, as they are distributed at equal intervals around the periphery of the nucleus, no two ever being in contact and the halves of each (bivalent) chromosome rarely separating. The chromosomes now evidently repel each other, while the halves of each chromosome attract each other rather strongly. The halves of these bivalent chromosomes are usually short rods, but they may be dumb-bell or hour-glass shaped, or nearly globular, as previously mentioned (12). Sometimes, however, this split fails completely to occur in the anaphase, the daughter chromosomes remaining single and globular or somewhat elongated (*fig. 39*). These telophase stages and the prophase of the homotypic mitosis will be taken up in detail in a paper dealing with different forms. These results, therefore, will not be duplicated here, but a brief statement of the events of the second mitosis will be given.

#### HOMOTYPIC MITOSIS

In the telophase of the heterotypic mitosis the nuclei never pass into the resting condition and the chromosomes never lose their identity completely, though they spread out and anastomose with each other more or less. Nucleoli are formed, as previously described (11). These stages between the two mitoses last for some time, but the events of the second mitosis are passed through very quickly. The two homotypic spindles are formed simultaneously and their axes are

at various angles to each other. Spindle formation is the same as for the heterotypic mitosis, except that the spindles are smaller. In regard to the chromatin, suffice it at present to say that the chromosomes of the homotypic prophase show the same general types and are often identical in appearance with those of the heterotypic telophase. There can be no doubt that the bivalent bodies which appear on the homotypic spindle are the same bodies that were present in the telophase of the heterotypic. *Fig. 41* shows an early anaphase of the second mitosis, the members of each pair having just separated. One of the small nucleoli appears by one of the spindles.

#### IRREGULARITIES

In *fig. 39* spindle fibers are seen in the cytoplasm by the side of the spindle in anaphase. This may be connected with a condition which is illustrated in *fig. 40*. Six such cases were observed in which a regular spindle occurred at the side of the mother cell instead of between the daughter nuclei, after the partial or complete disappearance of the heterotypic spindle. Some of these cases were in the telophase of the heterotypic spindle (*fig. 40*); others were in the prophase of the homotypic. In these cases the spindles were regularly formed and rather sharp-pointed and occupied the same position at the side of the cell; of course they contained no chromosomes. The method of their origin is unknown, but it seems probable that they are connected with the condition observed in *fig. 39*. Mother cells which probably indicate an intermediate condition, in which irregularly arranged fibers were found at the side of the cell, were occasionally observed. They may merely indicate a persistence of the kinoplasm of the heterotypic spindle after its function has ceased, but their structure appeared remarkably definite in most of the cases observed.

A single case of extra nuclei in the pollen tetrad was observed in *O. rubrinervis*. These have been previously described in *O. lata* (II), where they are common occurrences in connection with pollen degeneration. The single case observed in *O. rubrinervis* is sketched in *fig. 42*. Two small nuclei are present in addition to the four larger ones composing the tetrad. The nuclei had passed too far into the resting condition to count the chromosomes in each nucleus.

## POLLEN DEGENERATION

The general question of pollen degeneration in *Oenothera* is an interesting one. It reaches its extreme expression in *O. lata*, which is usually completely sterile in this regard, and in which I have already shown (11) that irregularities occur during the reduction divisions similar to those found in sterile hybrids. The question of sterility is evidently, as TISCHLER (32) suggests, a relative one.

In *O. rubrinervis* one is led from a gross examination to judge that the pollen production is copious and probably equal to that of *O. Lamarckiana* itself, but in reality many of the pollen mother cells fail to complete their divisions. From an examination of sections of anthers of *O. rubrinervis* it is found that in some loculi a large number or perhaps nearly all the mother cells may be degenerating in the synapsis stage. Frequently the cells are flattened and distorted, appearing pressed together for lack of space in the loculus. The chromatic contents of such cells often form a dense irregular mass, or their nuclei may be in normal synapsis or mitosis, notwithstanding the distorted shape of the cell; while still other cells of the same loculus may be entirely normal. Even earlier, in the archesporial stage, the tapetal cells in many sections were found to be breaking down, as in *O. lata* (11). No indications of degeneration have yet been observed in mother cells of *O. Lamarckiana*, and very few in the tapetum.

The percentage of mother cells which thus degenerate in *O. rubrinervis* was not determined. TISCHLER (32) suggests that the causes of sterility in mutants are the same as those in hybrids and in plants under cultivation. This general cause he designates as a disturbance or derangement of the constitution of the idioplasm, which he thinks has taken place in the production of mutants as well as in hybrids and under the conditions of cultivation.

## PROTOPLASMIC CONNECTIONS

It is an interesting fact that large and rather conspicuous protoplasmic connections are found between the pollen mother cells in *O. rubrinervis*. They are usually quite easily seen and it is probable that they are always present. They consist of delicate strings or threads of cytoplasm connecting adjacent mother cells. In size they vary greatly, from the delicacy of a spindle fiber to a coarse thread or

strand connecting the cells (*figs. 45, 46*). When the cytoplasm has shrunk slightly away from the cell wall they are particularly clearly observable. These connections appear to be in all cases between mother cells, and in no case have they been observed between the mother cells and the tapetum. Generally one such strand is seen connecting two cells, but not infrequently there are two or three or occasionally even more. There is no constriction or change in the nature of the connective as it passes through the cell wall. These connections are even larger and more conspicuous in *O. gigas*, where the mother cells are also much larger. They have not been observed in *O. Lamarckiana* or the other forms, but they doubtless occur in all, being probably smaller and more inconspicuous in some.

### Discussion

The method of reduction described in this paper at once raises a number of questions of prime importance from the cytological standpoint, as well as from that of the relation subsisting between hereditary and cytological phenomena. A discussion of all these features will not be attempted at this time, the intention of the writer being merely to indicate the general directions in which the facts point and the possible bearing which these data may be found to have on the problems connected with the phenomena of mutation in *Oenothera*. A fuller discussion of these subjects is reserved for a future time, after the presentation of further data. In the present communication reference will be made only to the most recent papers on reduction in plants, the purpose not being a review of the literature, or a discussion of present views, except in so far as they bear directly on the matter in hand.

The recent accounts of reduction in plants, given by BERGHS (3, 4, 5, 6), GRÉGOIRE (16), STRASBURGER (31), ALLEN (1, 2), MIYAKE (18), OVERTON (22), ROSENBERG (25), YAMANOUCI (33), and others, have agreed in so far as the following general course of events is concerned: In synopsis a pairing of homologous maternal and paternal elements occurs either in the form of gamosomes (STRASBURGER and MIYAKE), prochromosomes (OVERTON), or parallel threads (ALLEN, ROSENBERG, GRÉGOIRE, BERGHS, CARDIFF 7, and YAMANOUCI). In every case two parallel threads result, which unite

more or less intimately about the time of synapsis or later. After the events of synapsis, a longitudinal split reappears in the thickened spirem threads, this split representing the line of approximation of the two original spirems. Transverse segmentation into pairs of chromosomes, which are believed to be homologous somatic chromosomes of maternal and paternal origin, then takes place. The halves of these bivalent chromosomes, which lie side by side, are then distributed in the heterotypic mitosis, which is thus a reduction division. In the anaphase of the heterotypic mitosis a longitudinal split appears in the daughter chromosomes, which is regarded as a premature split for the homotypic mitosis, the latter being thus an equation division. The persistency with which this general account has been given, notwithstanding differences in detail, particularly preceding and during synapsis, leads the writer to the belief that it is probably correct in its main outlines, at least in many of the forms described. This being judged to be the case, every effort was made to bring the account in *Oenothera* into harmony with this general course of events but without success, for *Oenothera* is found to deviate in some important particulars, as is already evident from the description.

Another general account of reduction in plants, which was adhered to by STRASBURGER as late as 1904 (30), and has been held notably by FARMER and MOORE (8, 9), FARMER and SHOVE (10), SCHAFFNER (28), MOTTIER (19, 20), and others, to mention only a few of the recent papers, is in general as follows: The split in the spirem which occurs at about the time of synapsis is a true split, such as may occur in the prophase of somatic mitoses, and is not preceded by a pairing of parallel threads, but the thread is single from the beginning. This split afterward closes up as the thread shortens and thickens after synapsis, and the single spirem so formed segments usually into the reduced number of chromosomes, which are thus arranged successively end to end. Each such bivalent chromosome thus consists of two halves arranged end to end, not side by side, and the heterotypic mitosis thus separates successive whole chromosomes on the spirem, being therefore, as in the other account, a reduction division. The split which appears in the anaphase of this mitosis is interpreted as a reappearance of the earlier longitudinal split of the spirem. The homotypic mitosis is therefore an equation or longitudinal division.

There are of course minor differences in these accounts, SCHAFFNER (28) stating, for example, that in *Lilium tigrinum* there is a splitting of granules in the spirem, but the linin thread remains single. Differences of opinion are also expressed regarding the arrangement of the loops of the spirem before segmentation, and their relation to the chromosomes formed.

These two general schemes agree that the heterotypic mitosis is a reduction division separating whole somatic chromosomes, while the second division is longitudinal. The essence of the distinction is that the first view regards the chromosome bivalents as formed by a side-by-side union of homologous chromosomes through the medium of parallel threads, while the second view holds to an end-to-end union. It will be seen that, omitting the points which are left undetermined, the account in *Oenothera* corresponds more nearly with the latter scheme than with the former, though differing in some respects from both. ROSENBERG (25), from a comparison of forms having long and short chromosomes, has attempted to harmonize the latter view with the former. He examined *Listera*, *Tanacetum*, *Drosera*, and *Arum*, and found that, for example in *Drosera*, which has short definitive chromosomes much like those of *Oenothera*, the spirem first segmented into long twisted chromosomes lying in pairs with their long axes parallel. Later, as they condensed into the short, rounded definitive chromosomes, they frequently swung around end to end, so that an observer seeing only the later stage would conclude that they had been arranged tandem on the spirem at the time of their origin. Similar conditions were sometimes observed in *Listera*. I think my *figs. 22-28* make it evident that this explanation will not apply to *Oenothera*. The chromosomes in *Oenothera* do not undergo any such great amount of condensation, but are already thick, heavy bodies when first formed from segmentation of the spirem (*fig. 24*). Their diameter at this time is about the same as that of the spirem just previous to segmentation, as is shown by comparing *figs. 22* and *23* with *figs. 24* and *26*. The fact that as many as eight or more chromosomes may be found forming a single connected chain (*fig. 26*) also renders this explanation impossible.

MIYAKE (18) finds that after the pairing of elements in synapsis (the exact method of this pairing need not be entered into here) in

Galtonia and Tradescantia, a longitudinal split appears in the thickened thread, and the double spirem thus formed breaks transversely into the reduced number of chromosome pairs. Later, in these forms, a secondary union between the chromosomes is claimed to take place, forming a single connected chain of chromosomes (as in *Oenothera*). Sometimes a pair of chromosomes lies free by itself at this time. Then by further shortening the chromosomes of Galtonia again fall apart into pairs, though in Tradescantia they frequently remain connected even after spindle formation. The apparent similarity of the chromosome chain thus described by MIYAKE in Galtonia to the condition in *Oenothera*, led the writer to make an endeavor to harmonize the two accounts. But instead of this, all the evidence obtained from a critical study of the stages concerned shows that in *Oenothera* a single very thick spirem breaks transversely into the sporophyte number of chromosomes. A critical examination of *figs.* 22-28 will make it clear, I think, that we are following the progressive segmentation of a single spirem, and there is no room for stages between, in which a double spirem breaks into two parallel series of chromosomes. Moreover, it is hardly likely that secondary fusions between chromosomes would take place to such an extent as is shown in *figs.* 23 and 24. In nuclei such as *fig.* 20, in which a pair of chromosomes is cut off prematurely from the spirem while still in the second contraction, they are invariably connected at one end and rarely, if ever, at the other (though sometimes the close approximation of the latter ends may give the false appearance of a ring). This would not be the case if they came from separate paired threads merely lying side by side, so that this connection shows them to have been really successive on the spirem. From this evidence the writer cannot see how anything except a distortion of the facts can lead to the assumption in *Oenothera* of two parallel threads breaking into chromosomes. Hence the conclusion is that the double threads appearing in the stage represented by *fig.* 17 have united to form a single thread, which then breaks transversely into the sporophyte number of chromosomes.

This corresponds fairly well with STRASBURGER'S 1904 (30) account of the post-synaptic stages in Galtonia, and suggests to the writer that perhaps after all the earlier account may be nearer the facts,

so far as the points here under discussion are concerned, than the paper of 1905 (31). The close similarity of the conditions in *Galtonia* and *Tradescantia* during diakinesis to those in *Oenothera* suggests that they may be found finally to conform to *Oenothera* in these later stages. Whether or not this will be found to be the case, we must conclude that in *Oenothera* the longitudinal fission in the spirem (however it originated) closes up, and that after the second contraction, or during it, the thick thread segments into the sporophyte number of chromosomes. Since this diverges in important respects from nearly all the recent accounts of reduction in plants, the conclusion is that reduction probably takes place differently in different plants. Whether or not the results are different from the standpoint of a qualitative distribution will not be discussed now. The writer believes the above conclusions to be necessary, despite the fact that authors have reached different conclusions in regard to the same plant, particularly in such cases as *Lilium* and *Podophyllum*.

The next important point which requires discussion and which was left undecided in the statement of observations, is in regard to whether the double thread observed after synapsis arises from an approximation of parallel filaments or through a primary split in the thread. It may be well to examine the results which follow from either assumption. The writer hopes later to determine more definitely this difficult matter. On the first assumption of a lateral approximation in synapsis of two spirems representing respectively the maternal and paternal chromosomes, we should expect the double thread so formed to segment into the *reduced* number of chromosome pairs, in order to conform to the current account in forms in which there is a pairing of spirems, for example ALLEN (1), GRÉGOIRE (16), and YAMANOUCI (33). Instead, however, the spirem segments into the unreduced number of bodies. We may still assume that each of these bodies consists of maternal and paternal longitudinal halves still closely held together and resulting from a previous approximation. According to this view the first mitosis would separate bodies which were arranged successively on the spirem, while the second mitosis would separate the maternal and paternal halves of these bodies. The reason for such a result would be that the maternal and paternal spirems remained closely fused after pairing, so that

their elements were separated in the second mitosis instead of the first. This view is scarcely admissible for several reasons. In the first place, on this hypothesis transverse segmentation of the spirem must have taken place not only between the (bivalent) chromosomes but also in the middle of each chromosome, in order to give a chain of fourteen bodies. Such a segmentation seems unlikely. Another possible explanation would be that the chromosomes have lost their identity during synapsis, and that the bodies we are dealing with now are new arrangements of the chromatic material, irrespective of the somatic chromosomes. Many considerations, however, strongly support the belief that these bodies really represent the somatic chromosomes. The facts so far educed in *Oenothera*, in the opinion of the writer, all favor the hypothesis of the separate existence and genetic continuity of the chromosomes from one generation to another. In this connection may be cited certain plants from the  $F_1$  of *O. lata*  $\times$  *O. gigas*, which as stated elsewhere (14) have 21 chromosomes as somatic number, 10 of which regularly go to one pole of the heterotypic spindle and 11 to the other. Occasionally, however, the segregated numbers of chromosomes are 12 and 9, one chromosome having gone to the wrong pole of the spindle. In this hybrid 7 of the chromosomes are maternal and 14 paternal. If in this case there were a pairing of maternal and paternal spirems, it is difficult to see how it could be accomplished and result in the distribution of chromosomes in the heterotypic mitosis already stated.

It will be instructive to compare the chromosome history in this cross with the often-quoted condition found by ROSENBERG (23, 24) in *Drosera longifolia*  $\times$  *D. rotundifolia*. *D. rotundifolia* has 10 chromosomes and *D. longifolia* 20, as the gametophyte number. The hybrid naturally has 30 chromosomes in its sporophyte tissues, but in diakinesis 20 chromosome bodies appear, 10 of which are double, consisting of a larger and a smaller half, while the remaining 10 are the unpaired (smaller) *longifolia* chromosomes. The larger and smaller halves of the 10 bivalents separate and pass regularly to the poles of the heterotypic spindle, but the unpaired chromosomes are irregularly distributed or left out of the daughter nuclei. Later the pollen deteriorates. This result is strikingly different from that in the *Oenothera* hybrid, and, while perfectly in harmony with the

idea of the pairing of threads in synapsis in *Drosera*, makes it highly probable, and in fact necessary, that the method of reduction in the *Oenothera* hybrid be different. This is a strong argument not only against pairing of maternal and paternal spirems in *Oenothera*, but in favor of the probability that reduction takes place in diverse ways in the two genera. A considerable amount of time has already been devoted to the study of reduction in this *Oenothera* hybrid, and an account will be published later. So far as observed it shows no differences in method from the account given here for the pure races.

The hypothesis of the pairing of parental spirems in synapsis in *Oenothera* being thus rejected, the other alternative remains, namely, that the double spirem results from a split; and this appears to satisfy all the facts. The observations have already shown that the spirem segments into a single chain of chromosomes. The description of events in *Oenothera* from synapsis on thus agrees in outline with the 1904 account of STRASBURGER (30) in *Galtonia*, and in general also with that of FARMER and MOORE (9) in *Lilium*, *Osmunda*, *Psilotum*, and *Aneura*, FARMER and SHOVE (10) in *Tradescantia*, and MOTTIER (19, 20) in *Lilium*, *Podophyllum*, and *Tradescantia*. The belief of the writer is that some of these forms will be found to correspond more nearly with the account which involves a pairing of threads, and some with the account involving only a split.

Another important matter which requires mention at this time is the nature of the chromosome distribution which takes place on the heterotypic spindle in *Oenothera*. As already observed, the chromosomes even during spindle formation are frequently unpaired. This appears to be due to the weakness of the mutual attraction which ordinarily leads to pairing. Granting that homologous maternal and paternal chromosomes unite when pairing takes place, what are the possibilities regarding the unpaired chromosomes? Pairing insures ordinarily that the members of the pair will proceed to opposite poles of the spindle, and hence that the homologous maternal and paternal elements will enter different nuclei. There is no such certainty in the distribution of the unpaired chromosomes, so that it might be expected that in certain cases both members of a pair would enter the same daughter nucleus. It is important to note that this result is entirely independent of the origin of these chromosome

pairs, whether from an end-to-end or side-by-side union of somatic chromosomes, or in any other manner, so that this question holds no necessary relation to the method of reduction. On the common cytological assumption that the chromosomes are qualitatively different (which has apparently been shown to be a fact in certain well-known cases in animals, that need not be cited), germ cells would occasionally arise lacking both members of a pair, and hence lacking the possibility of developing certain qualities. In this manner it is conceivable that a series of types might arise from the parent *O. Lamarckiana*, each lacking the possibility of developing a certain group of characters possessed by *O. Lamarckiana*.

On this view, which is suggested merely as a tentative hypothesis, we would have in the mutations of *O. Lamarckiana* an analytical process in which a series of types arises from the parent form, each lacking in a different group of qualities or capacities which the parent form possessed. This does not apply to *O. gigas*, however, which will be taken up at another time. The further bearings of this hypothesis on the mutation theory of DEVRIES will not be followed up in this discussion, but it may be pointed out here that such a hypothesis accounts for the absence of reversions of the mutants to *O. Lamarckiana*, and it may also account for some of the peculiarities of hybridization among the *Oenothera* mutants. I should therefore suggest that there may be a relation between the type of reduction in any organism and its variation and hybridization phenomena.

In *Galtonia* and probably also in *Tradescantia* there are apparently the same possibilities that both chromosomes of a pair may occasionally enter the same daughter nucleus. In other plant forms studied the attraction between chromosomes seems to be strong enough to keep the members of a pair together until their separation in the anaphase of the heterotypic mitosis. The segregation of the members of a pair into separate germ cells is thus insured. In cases where, as in *Oenothera*, the members of a pair do not always remain in contact, but are loosely arranged on the spindle, such a result as already suggested seems certain to occur in certain instances.

It has already been mentioned that occasionally one chromosome goes to the wrong pole of the heterotypic spindle. This is found to be the case particularly in the hybrids, for example, in the *O. Lamarcki-*

*ana* plants from the  $F_1$  of *O. lata*  $\times$  *O. Lamarckiana* (13), in which sometimes eight chromosomes pass to one pole and six to the other; but it may also occur rarely in the pure races. This matter was briefly discussed elsewhere (14). Assuming that the 14 chromosomes are in two similar sets of 7 each, and that homologous members of these sets conjugate except when there is a failure to pair, then when 8 chromosomes go to one pole and 6 to the other, both members of one of the pairs must have gone to the same pole. This probably takes place in cases where such members were unconjugated, for the purpose, or at any rate, the result of the pairing is in ordinary cases that one member of every pair shall be distributed to each pole. If, while two members of one pair thus go to one pole, the second member of another pair goes to the other pole, we should have an equal numerical distribution of chromosomes, but one daughter group would be lacking both members of one pair and the other would be lacking both members of another pair. It is highly probable that such a distribution occasionally takes place, though it would be less common than the case, already proved, where the members of a single pair are unilaterally distributed. It should be borne in mind that such cases are most likely to occur, not when the members of a pair are conjugated, but when they lie separately in diakinesis and on the spindle.

Miss LUTZ (17), from an examination of root tips, states that she has observed several individuals belonging to different strains having 15 chromosomes instead of 14. This is to be anticipated from the irregularities in chromosome distribution in reduction already mentioned. I have observed one such case in *O. lata*  $\times$  *O. gigas* (14)—a certain plant having 20 chromosomes instead of 21. All the plants of *O. lata* (12) and *O. nanella* (13) thus far examined by me had 14 chromosomes, while Miss LUTZ (17) finds in root tips some *O. lata* plants with 14 and also some with 15 or she thinks possibly 16 chromosomes. She reports finding two *O. nanella* plants with 14 chromosomes and one with 15. Two *O. albida* seedlings are said to have 15 chromosomes and two *O. oblonga* plants 15, while a third has 14. Disregarding the possibility that these results might be due to the well-known variation in chromosome numbers in root tips, they are such as would be likely to arise in different individuals from the cytological irregularities I have already described. Whether there

are external differences between the plants having 14 chromosomes and those of the same race having 15, is as yet unknown. But it is quite conceivable that no such differences will be found, for if the sporophyte chromosomes consist of two complete sets (and for a variety of reasons this seems the only tenable view at the present time if we assume qualitative differences at all), the presence of an additional chromosome, which is already present in duplicate, would scarcely be expected visibly to affect the plant.

ROSENBERG (26) has found an analogous situation in *Hieracium*. For example, *H. excellens*  $\times$  *H. Pilosella* gives hybrids with different numbers of chromosomes. This he ascribes to the fact that the eggs of *H. excellens* differ in their numbers of chromosomes, which he finds is due to irregularities in chromosome distribution during the reduction divisions. The writer has pointed out elsewhere (12) certain similarities between the hybridization phenomena in *Hieracium* and *Oenothera*, and this seems to be a further similarity between the two genera.

ROSENBERG (27) has since shown that *H. excellens* produces three kinds of embryo sacs: (1) Normal embryo sacs which require fertilization for their development. These are presumably the only ones which can be hybridized. The egg cells in these sacs vary in their number of chromosomes owing to the fact that some of the chromosomes, lacking in "affinity," remain univalent (that is, fail to pair) during the heterotypic mitosis and are irregularly distributed. It is evident that this lack of affinity between chromosomes is similar to that in *Oenothera*. (2) In rare cases apogamous embryo sacs are formed after a single division of the megaspore mother cell, and without reduction. (3) More frequently the condition occurs which ROSENBERG calls apospory, in which tetrad formation takes place and then an adjacent cell of the nucellus enlarges, displaces the tetrad, and forms an embryo sac without reduction.

### Summary

In conclusion a brief summary of the facts and considerations here presented will be useful.

1. In *Oenothera* the heterotypic mitosis is a reduction division, separating whole chromosomes which lie successively on the spirem. The homotypic mitosis is an equational division, separating the longi-

tudinal halves of the daughter chromosomes of the heterotypic mitosis. Whether an approximation of threads or a split in a single thread occurs in synapsis was not determined with certainty from the observations, but various considerations lead to the belief that in *Oenothera* the doubling is due to a split which closes up later, rather than to an approximation of separate spirems.

2. The conclusion that the method of reduction probably differs in different genera is based on two considerations: (1) the fact that in most of the recent accounts of synapsis and reduction in plants a side-by-side pairing of chromosomes from maternal and paternal spirems is described, while in *Oenothera* the members of a pair are arranged end to end on a single spirem; and (2) on differences in chromosome distribution during reduction in certain hybrids of *Drosera* and of *Oenothera* (see p. 25). If reduction took place in the same manner in both genera, the chromosome distribution during reduction in these hybrids with reference to the parental chromosome numbers should be the same in both, but this is not the case.

3. Pairing between the definitive chromosomes during diakinesis and the prophase of the heterotypic mitosis does not always take place, owing to a weak attraction between the chromosomes. This allows irregularities of distribution in the heterotypic mitosis, so that both (unpaired) chromosomes belonging to one pair will *occasionally* enter the same daughter nucleus (see p. 26). Germ cells will thus arise, from which both members of a given pair of chromosomes are absent.

4. If we assume qualitative differences between the chromosomes or parts of them, various types would be expected to originate in this manner, each of them lacking the ability to develop certain qualities possessed by the parent form. On this view the mutations of *Oenothera Lamarckiana* are an instance of a process of analysis by which from the parent form arises a series of types, each lacking in certain characters or capacities possessed by the parent. This hypothesis would account for the absence of reversions among *Oenothera* mutants, and perhaps also for some of the peculiarities of hybridization in *Oenothera*. This matter will be considered at another time. This explanation does not apply to all the mutants, however; for example, *O. gigas*.

5. It is suggested that there is probably a direct relation between the events of reduction in a given genus and its variation, as well as its hybridization phenomena.

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### EXPLANATION OF PLATES I-III

The figures were drawn with the aid of a Bausch & Lomb camera lucida. All except *figs.* 1 and 19 were drawn under a Zeiss apochromatic objective 2<sup>mm</sup> ap. 1.30, with a Zeiss compensating ocular 18. The figures are reduced one-fourth in reproduction, giving a magnification of nearly 3000 diameters. *Fig.* 1 was drawn under a 2<sup>mm</sup> objective and compensating ocular 6; *fig.* 19 under B. & L. objective  $\frac{1}{16}$  N. A. 1.32 and Zeiss ocular 18.

## PLATE I

FIGS. 1, 2.—Young meristematic cells of anther primordium showing one large nucleolus and several smaller ones, and chromatic masses adherent to the nuclear membrane.

FIG. 3.—Longitudinal section of anther, showing size relations of nucleoli in sporogenous, tapetal, and wall cells.

FIG. 4.—One sporogenous cell from stage of *fig. 3*, previous to synapsis; cytoplasm somewhat vacuolate.

FIGS. 5, 7-9.—Nuclei at same stage, showing fusions of nucleoli.

FIG. 6.—Two nucleoli of equal size; an unusual condition.

FIG. 10.—Several small nucleoli, and no indication of fusion.

FIG. 11.—Nucleoli of young pollen grain nucleus.

FIG. 12.—Beginning of synaptic contraction; the reticulum has contracted from the nuclear membrane on all sides, leaving several loops attached to the membrane; on the side on which the reticulum retains the curved outline of the nuclear membrane the latter has been drawn inward attached to the threads; on the rest of the circumference, between the loops, the nuclear membrane remains *in situ*; the cytoplasm is perfectly fixed.

FIG. 13.—Another contraction stage, showing loops attached to the nuclear membrane, which is intact.

FIG. 14.—A slightly later stage of contraction, in which the rearrangement of threads is taking place.

FIG. 15.—Synapsis; dark-staining bodies are still held in the meshes of the spirem; a small nucleolus, usually about the size of a chromosome, is generally present in addition to the large nucleolus.

FIG. 16.—After synapsis; the thread thicker and shorter and loosely coiled.

FIG. 17.—Slightly later stage than *fig. 16*, and less deeply stained; thread shows the characteristic light and dark areas; indications of parallel threads in two places; edge of thread may be even or moniliform.—5  $\mu$ .

FIG. 18.—Later stage; thread much shortened and greatly thickened and entering upon second contraction phase; nucleus uncut.—10  $\mu$ .

FIG. 19.—Higher magnification of a portion of the thread in *figs. 20* and *21*.

## PLATE II

FIG. 20.—Second contraction stage; a pair of chromosomes cut off from spirem; nucleus uncut.—10  $\mu$ .

FIG. 21.—Second contraction stage; nucleus uncut.

FIG. 22.—Uncoiling from second contraction stage; pair of chromosomes detached; nucleus uncut.

FIG. 23.—Spirem segmented in three places, each segment showing constrictions which will form the chromosomes; certain chromosomes already detached; nucleus uncut.

FIG. 24.—Constriction of spirem has proceeded farther, the chromosomes being elongated bodies with irregular margins like the spirem, and connected by rather

thick "linin" bands; pair of chromosomes detached earlier lies at side of nucleus; *n*, small nucleolus; nucleus cut.

FIG. 25.—Spirem more or less completely segmented into chromosomes while still in the second contraction stage; preparation considerably destained; 13 chromosomes in view.

FIG. 26.—Spirem segmented, showing chain of eight chromosomes and three pairs; nucleus uncut.

FIG. 27.—Chain of six chromosomes, and probably four pairs; linin connections between members of a pair not always visible; nucleus uncut.

FIG. 28.—Fourteen chromosomes; several small nucleoli; nucleus uncut.

FIG. 29.—Fourteen chromosomes, including five pairs more or less closely associated; linin connections not visible; one pair of chromosomes has already contracted into the globular shape.

FIG. 30.—Fourteen chromosomes, several in pairs; apparent inequalities in size due to positions in which some of the chromosomes are lying.

FIG. 31.—Slightly later stage; the fourteen chromosomes contracted into the globular or pear-shaped definitive form; linin connections longer and extremely delicate; nucleus uncut.

FIGS. 32-34.—Other groups in diakinesis, showing various peculiarities of chromosomes.

FIG. 35.—Peculiar case of spindle formation; three nucleoli present and fourteen chromosomes, including three or four pairs.

FIG. 36.—Multipolar stage of heterotypic spindle; two more or less closely united pairs of chromosomes present.

#### PLATE III

FIG. 37.—Same as *fig. 36*; an unusual case in which all the chromosomes are closely joined in pairs; seven such pairs present and a small nucleolus.

FIG. 38.—Heterotypic spindle in metaphase; spindle has usually more mantle fibers than in *O. Lamarckiana*; chromosomes usually loosely arranged in equatorial region of spindle.

FIG. 39.—Late anaphase; an uncommon case; daughter chromosomes have failed to divide, and fibrillae are scattered in cytoplasm at side of cell; chromatic staining material also present.

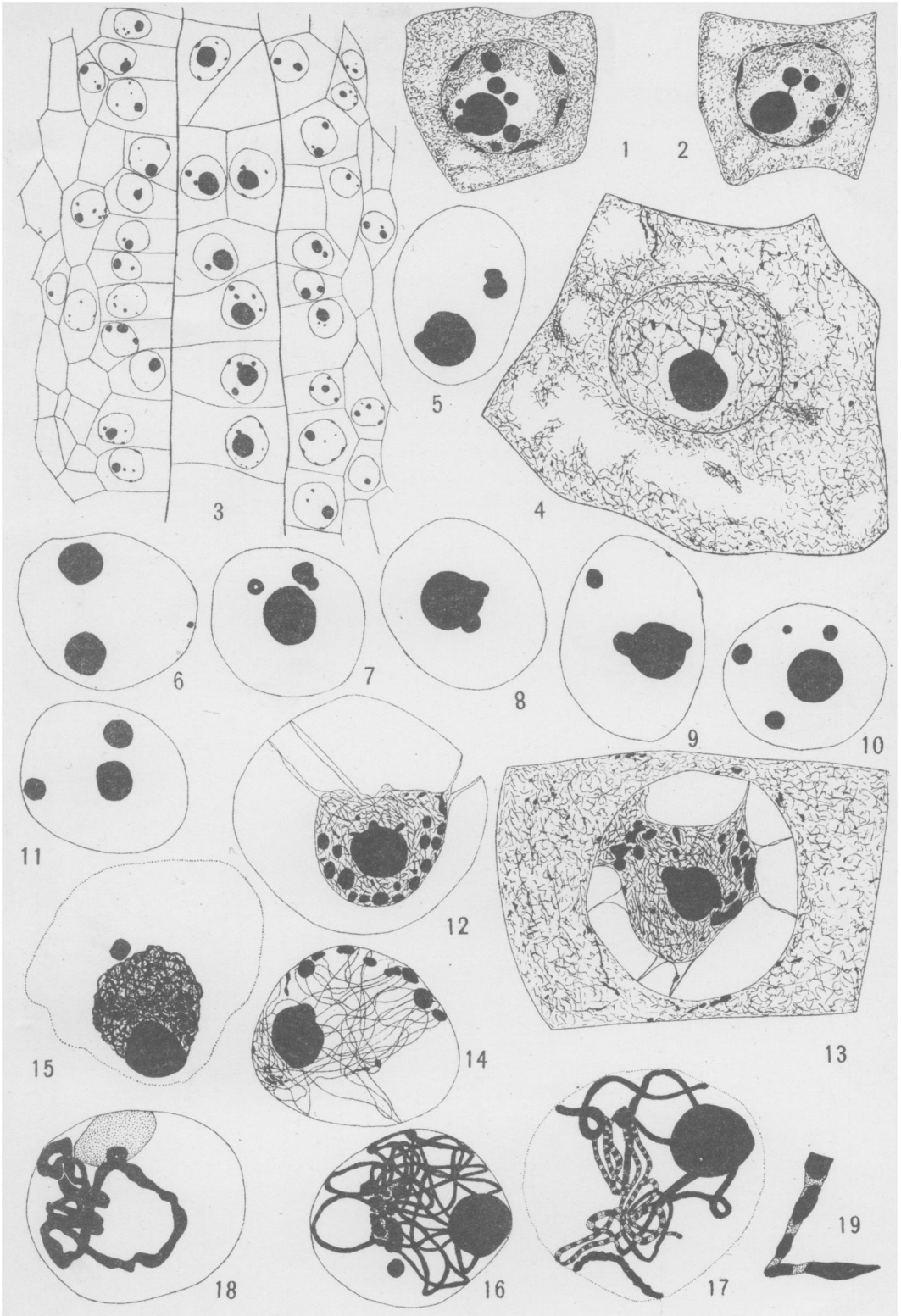
FIG. 40.—Telophase of heterotypic mitosis; exceptional case, in which a rather sharp pointed spindle is formed at side of cell; it probably originated from the fibrillae shown in *fig. 39*.

FIG. 41.—Early anaphase of homotypic mitosis; small nucleolus having the characteristic appearance, present on one of the spindles.

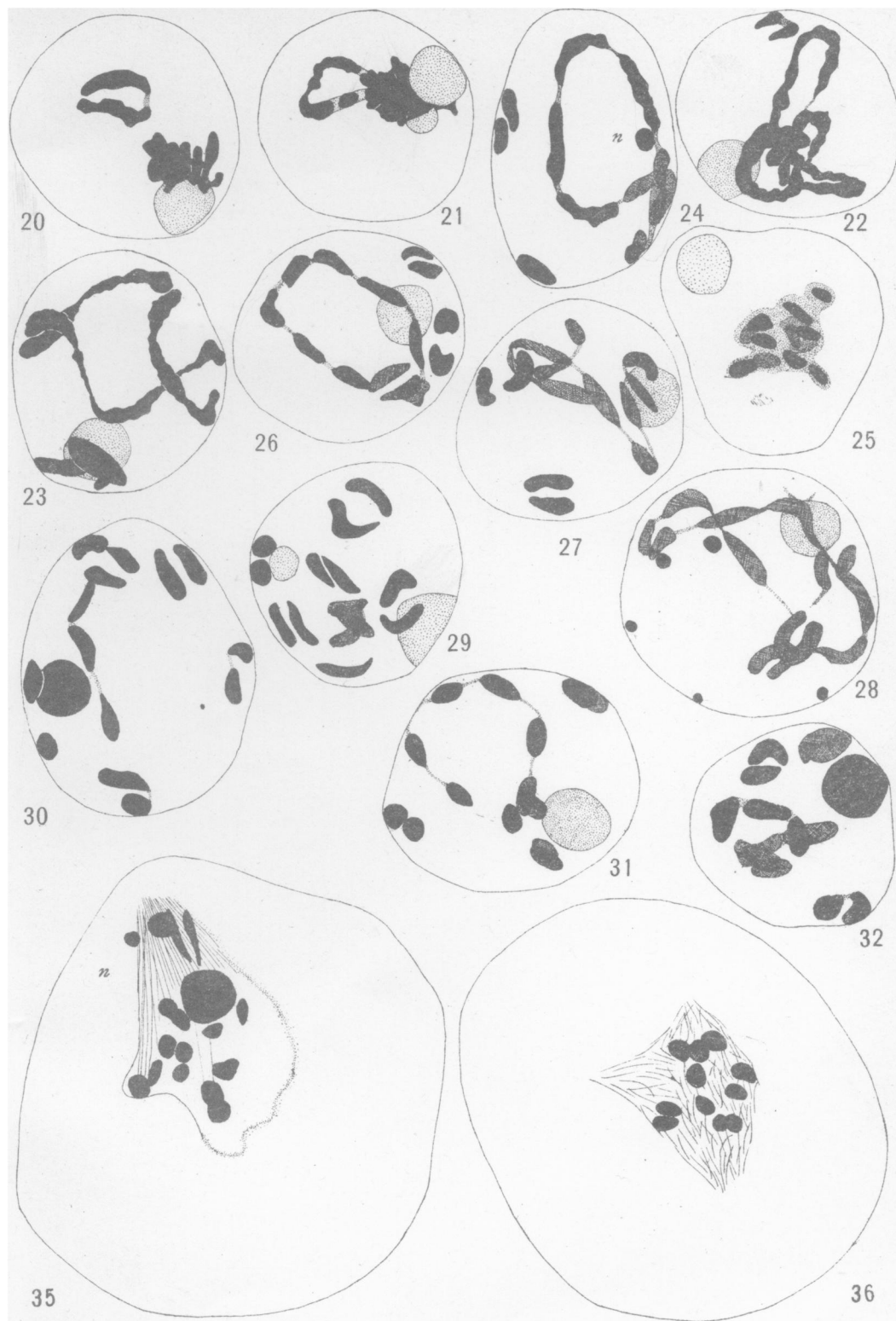
FIG. 42.—The single case of extra nuclei observed in *O. rubrinervis* pollen mother cells.

FIGS. 43, 44.—Nuclei from telophase of second mitosis, passing into resting condition.

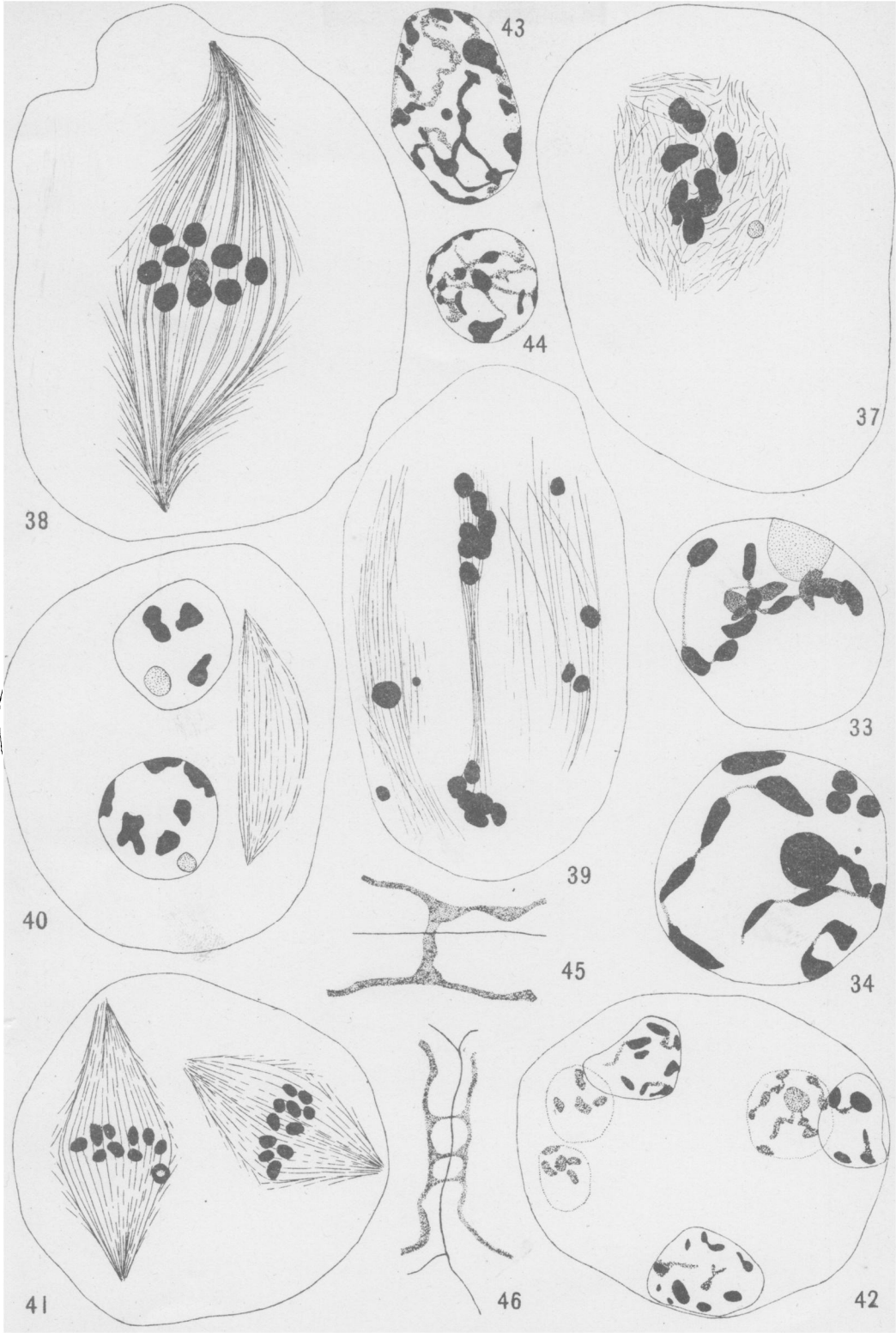
FIGS. 45, 46.—Protoplasmic connections between mother cells.



**GATES on REDUCTION in OENOTHERA**



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